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FEMALES INHIBIT MALES' PROPENSITY TO DEVELOP INTO SIMULTANEOUS HERMAPHRODITES IN *CAPITELLA* SPECIES I (POLYCHAETA)

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Abstract

The propensity for a male of *Capitella* species I to develop into a simultaneous hermaphrodite is shown to be inhibited by the presence of females. However, even when females are rare, males which develop into hermaphrodites do so nearly as quickly as males held in all male cultures or in isolation. When held in isolation males which do not switch are smaller than their male siblings which do. The maternal parent has no effect on the propensity of switching; no differences are found between male offspring which are derived from either females or hermaphrodites. It has been suggested that hermaphroditism in *Capitella* is an adaptation to low density. Yet females do not become hermaphroditic, hermaphrodites do not self-fertilize, and hermaphrodites function primarily as females. It seems much more likely that hermaphroditism in *Capitella* is an adaptation to living in small local populations which experience local mate competition.

Introduction

The polychaete species *Capitella capitata* is known to actually be comprised of a large group of unnamed sibling species (Grassle and Grassle, 1976; Grassle, 1980) which exhibit a great deal of diversity in life history and sexuality. In one species, designated *Capitella* sp. I (Grassle and Grassle, 1976), natural populations are a mixture of males, females, and hermaphrodites which arise from males that have secondarily developed eggs. Hermaphrodites can function as either sex and offspring are the result of sexual reproduction (Holbrook, 1982). Since *Capitella* sp. I rapidly colonizes a disturbed habitat (Sanders *et al.*, 1980), the occurrence of hermaphroditism in the species has been linked to its opportunistic life history (Grassle and Grassle, 1976; Grassle, 1980; Holbrook, 1982; Holbrook and Grassle, 1984).

Recently, it has been shown that sex determination in *Capitella* sp. I is a simple two-factor system (Petraitis, 1985). Under careful rearing of progeny from single crosses with less than 20% mortality, females produce a 1:1 sex ratio while hermaphrodites which function as females produce nearly all male offspring. All female offspring from hermaphrodites are protogynous hermaphrodites and secondarily develop male copulatory setae. The simplest explanation is that males (*i.e.*, hermaphrodites) are the homogametic sex.

Holbrook and Grassle (1984) found that male offspring from broods with either "high male" or "low male" sex ratios show no difference in their propensity to develop into hermaphrodites. The first objective of this research was to examine for differences between male offspring from females and hermaphrodites as maternal parents.

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A second objective was to explore alternative explanations for the adaptive significance of hermaphroditism in *Capitella* sp. I. Holbrook and Grassle (1984) call the development of hermaphroditism an "emergency adaption" to low density. Holbrook (1982) suggests that the presence of females or a female pheromone suppresses ovum development in males. The low density model (Tomlinson, 1966; Ghiselin, 1969; Charnov, 1982) predicts that individuals which can develop into simultaneous hermaphrodites when mates are rare will be at a selective advantage. The observations that *Capitella* sp. I females do not become simultaneous hermaphrodites, that hermaphrodites cannot self-fertilize, and that older males will become hermaphrodites even at high density are not explained by the low density model.

As an alternative explanation, I propose that hermaphroditism in *Capitella* may be the result of mate competition. Since *Capitella* often colonizes areas over a short time period, I hypothesize that mating may be highly synchronized as most females reach maturity at the same time. As females are mated and begin to brood eggs, the ratio of receptive females to males would decline. At this point a male which could develop eggs and function as a female would be favored. If colonizers of a patch are closely related, competition among siblings for mates would have the same effect (Taylor, 1981). Thus one would predict some males should develop eggs when females are rare, regardless of the density. The second objective was to test this prediction.

Materials and Methods

All worms are from a stock culture started in the fall of 1980 with offspring from *Capitella* sp. I females from Woods Hole (Petraitis, 1984). For the last two years, stock cultures have been maintained at 15°C. Procedures for maintenance are identical to methods given in Petraitis (1984) except that worms older than three weeks are fed finely chopped frozen spinach in a bed of fine (less than 300 μm) sand. All experiments were done between March 1983 and February 1984.

In order to control for differences among families, full sibling offspring from a single cross were treated as experimental blocks. Offspring from each cross were raised in a single 4 inch culture bowl until they were used for an experiment. Sibships were then subdivided and used in each treatment.

A few broods which were directly drawn from the stock culture were used. The maternal parent was always female in these cases. Female and hermaphroditic offspring from the original broods were raised in isolation and then crossed with males from stock culture. Male offspring from the female X male crosses were used in experiments as "female-derived" males. Male offspring from the hermaphrodite X male matings provided "hermaphrodite-derived" males.

I examined the propensity of sex change in male offspring from hermaphrodites and females in an isolation experiment similar to the experiment described by Holbrook and Grassle (1984). From each of 15 sibships from hermaphrodite X male matings and 11 from female X male matings, 20 males were randomly chosen, and each worm was singly placed into a small petri dish (0.05 worms/cm^2). Worms ranged from 42 to 60 days in age. Water and food was changed twice a week. At the end of three weeks the number of males which had developed eggs were tallied. The length of all worms was measured with an ocular micrometer. As a check on length as a measure of body size, 100 worms were randomly drawn from the stock culture, measured, and then dried and weighed. The correlation between dry weight and length is +0.94.

Observations were also taken on cohorts of worms from the same sibships which were used in the isolation experiments. A group of 25 larvae from each sibship was placed into a 4 inch culture bowl. Bowls were checked every week for females and hermaphrodites with brood tubes. Tubes were removed, offspring were counted, and mothers returned to bowls. Every three weeks the bowls were censused and 10 worms were measured. Data were collected for life table analysis which will not be discussed here, however information on the occurrence of hermaphrodites is revelant and is discussed. Twenty broods from hermaphrodite X male crosses and 19 broods from female X male crosses were used.

Two experiments were conducted to test if the initiation of ovum development in males was affected by the presence of females. For the first experiment male offspring from a brood were randomly assigned to four treatments: a pure male treatment of 6 males per petri dish (0.29 worms/cm^2), a 50:50 sex ratio treatment of 3 males and 3 females per dish, a biased sex ratio treatment of 1 male and 5 females per dish, and a control of 1 male per dish (0.05 worms/cm^2). I set up two or three controls per sibship and one was randomly chosen for analysis. Progeny of a single brood thus formed a "block" and data were analyzed with Friedman's test (Sokal and Rohlf, 1981). If worms died, that block was not used. The sibships ranged from 35 to 69 days old with an average of 48 days at the start of the experiment. Dishes were maintained for 56 days or until at least one male in each treatment dish had developed ova. Dishes were checked at least twice a week. Thirteen blocks were successfully run. The second experiment was simply a continuation of the first except that an additional treatment of 5 males and 1 female per dish was added. This experiment used progeny from seven different broods.

Results

When held in isolation, males from hermaphrodite X male and female X male matings show no significant difference in the development of ova. All 20 male siblings developed ova in 8 of the 15 hermaphrodite-derived sibships and in 3 of the 11 female-derived sibships. The average percentage is 91% for hermaphrodite-derived males and 79% for female-derived males. These means are based on untransformed data; a *t*-test for difference between means, which were calculated with data transformed by the arcsine of the square root, is not significant ($t = 1.57$, d.f. = 24). Transformed means and standard deviations are 78.71 ± 15.51 for hermaphrodite-derived males and 69.93 ± 19.44 for female-derived males. Survivorship is quite good in both groups with 453 of the 520 males surviving. Mean survivorship is 17.9 out of 20 for hermaphrodite-derived males and 16.8 out of 20 for female-derived males.

Isolated males which develop ova are slightly larger than their male siblings which do not (Table I). When results of both types of sibships are combined, males which develop ova average 13.97 mm in length while their siblings are 11.38 mm. Since the observations are blocked by sibships, a paired *t*-test was used, and the difference is significant. Offspring from both types of sibships show the same trend, although the difference in hermaphrodite-derived families is not significant. This is probably due to small sample size.

The cohort observations are quite different. Hermaphrodites develop in 17 of the 20 cohorts from hermaphrodite X male matings and in 3 of the 19 cohorts from female X male matings. Hermaphrodites first appear in the bowls between 6 and 21 weeks; the average is 13 weeks. Males which develop into hermaphrodites are no bigger than their male siblings (Table I). Males from the cohort bowls are

TABLE I

Mean lengths of males which developed into hermaphrodites and of their male siblings which did not

	Length of siblings which are		Difference between pairs of observations
	herm.	male	
Isolation observations			
hermaphrodite-derived sibships (6)			
mean	11.36	9.06	2.31
S.D.	0.87	2.67	2.21
female-derived sibships (8)			
mean	9.86	8.18	1.69*
S.D.	0.68	1.65	1.37
combined (14)			
mean	10.51	8.56	1.95*
S.D.	1.07	2.10	1.73
Cohort observations			
hermaphrodite-derived sibships (17)			
mean	12.74	12.23	0.51
S.D.	3.32	2.56	1.98
female-derived sibships (3)			
mean	15.00	12.87	2.13
S.D.	3.00	1.63	1.63
combined (20)			
mean	13.08	12.32	0.75
S.D.	3.30	2.42	1.99

Means and standard deviations (S.D.) are given in units from the ocular micrometer scale. One unit = 1.33 mm. Numbers in parentheses are the number of sibships used in each case. Since males and hermaphrodites are paired by sibship, the mean difference is given. Significant paired *t*-test at the 5% level is denoted by asterisk.

larger than their male siblings held in isolation, however this is not surprising since males held in isolation were measured when they were about 10 weeks old while males held in cohorts were measured when they were usually older and thus larger.

Sex ratio, when density is held constant, has a strong effect on the initiation of ovum development (Table II). Males held in pure male dishes develop ova as frequently as males held in isolation. Indeed it appears that males held in pure male dishes develop ova more quickly. However note the means in Table II are calculated without taking block (*i.e.*, progeny from a single cross) effects into consideration.

For ten of the blocks in experiments 1 and 2, hermaphrodites appeared in both the pure male treatment and the control. When the difference between the control and treatment for these families is taken, males in pure male dishes take an average of 2.8 days longer to develop into hermaphrodites (S.D. = 6.2 days). The difference is not significant.

Since the difference between the treatments and the control may reflect density effects, treatments with females should be compared against the pure male treatment. When the pure male treatment value for each block is substracted from other treatment values, it is clear that the presence of females depresses the initiation of

TABLE II

Mean and standard deviation of the number of days required for the first male to develop ova under different sex ratios

	Males/females per dish				
	6/0	5/1	3/3	1/5	1/0
Expt. 1.					
mean	18.6		29.6	38.7	21.6
S.D.	5.8		8.7	4.9	7.3
percent	84.6		46.2	15.4	100.0
rank	1.5		3.4	3.5	1.7
Expt. 2.					
mean	27.0	24.8	35.0	29.0	17.0
S.D.	12.7	2.5	7.1	10.5	4.9
percent	71.4	42.9	28.6	42.9	100.0
rank	2.3	3.4	4.1	3.8	1.1

Row labelled "percent" gives the percentage of sibships in which at least one male did switch. "Rank" row is mean rank which is used in Friedman's test. Test is significant for both experiments. For experiment 1., $H = 26.75$, $df = 3$, $P \leq 0.001$ and for experiment 2., $H = 11.74$, $df = 4$, $P \leq 0.05$.

ovum development in males (Table III). One female in the presence of five males however is not sufficient to affect the rate.

There are no differences between hermaphrodite-derived and female-derived males in either likelihood or speed of sex change. When the observation of all control dishes are analyzed as a nested analysis of variance (Sokal and Rohlf, 1981), there is no significant difference between origin of male or among siblings within type of cross (Table IV). The likelihood of switching is also nearly identical. Under the pure male treatment 83% of the broods from hermaphrodite X male matings and 78% of the broods from female X male matings show the development of at least one hermaphrodite per dish.

DISCUSSION

Holbrook and Grassle (1984) found that males held in isolation required an average of 22 days to develop ova (their Table II) and suggest that not only the

TABLE III

The difference in days of each treatment minus the "pure male" (i.e. 6/0) treatment

	Males/females per dish		
	5/1	3/3	1/5
mean	−2.3	10.7*	14.8*
S.D.	10.7	6.2	14.3
n	3	7	5

Results are combined for both experiments shown in Table II, and the variate used to calculate the mean and standard deviation is the difference between treatments within sibships. The sample size, n, is the number of sibships in which at least one male changed sex in both the pure male treatment and the treatment under consideration. Paired *t*-tests which are significant at the 5% level are denoted by an asterisk.

TABLE IV

Nested analysis of variance of the number of days required for a male to develop into a hermaphrodite when held in isolation

Type of mother	Statistics for each sibship used in analysis					
Female						
mean number of days	27.3	21.0	19.5	16.5	13.5	19.5
standard deviation	2.9	0.0	5.0	3.5	6.4	7.8
sample size	3	2	2	2	2	2
Hermaphrodite						
mean number of days	20.3	21.0	19.0	18.5	15.0	
standard deviation	6.8	0.0	0.0	7.8	0.0	
sample size	3	3	3	2	2	

Source of variation	Analysis of variance		
	df	MS	F
Between type of mother	1	7.54	
Among sibships within type	9	35.86	1.75
Among progeny within sibships	15	20.52	

Data are control dishes (*i.e.*, 1/0 treatment) from experiments shown in Table II. Analysis of variance is not significant.

absence of females but also an excess of food may be required for development of ova by males. My result for the rate of development is quite similar; males develop ova in 20 days (Table IV). While my isolation experiments show that males which develop ova are larger than their brothers, this difference is not seen in males held in groups (Table I). It should be noted that larger size of the sex changers in the isolation experiment may have little to do with food availability. It is just as plausible that smaller males should be expected to be sex changers if they are shunting available energy into egg production rather than growth. At this point the biological significance of the difference in size is not clear.

Presence of females clearly inhibits the initiation of ovum development in males. In the presence of a single female, males developed into hemaphrodites in only 3 of the 7 experimental blocks (Table II), while combined results of experiments 1 and 2 show 16 of the 20 pure male treatment dishes produce at least one hermaphrodite. Since density and age are controlled, I infer the difference is due to the presence of a female. Data in Tables II and III suggest the presence of females affects the likelihood of switching more strongly than the number of days.

Holbrook and Grassle (1984) found no difference between males from broods with "high male" and "low male" sex ratios in the proportion of males which develop eggs (their Fig. 2). I found no difference between hermaphrodite-derived and female-derived males. I suspect their high male broods are in fact derived from hermaphrodites and their low male broods from females. They report 3 high male broods with a total of 161 males, 12 females, 25 unsexed juveniles, and 52 deaths before sexing. Their 3 low male broods have 96 males, 117 females, 21 unsexed juveniles and 96 deaths before sexing. Assuming no sex-specific mortality, high male broods are 93% male and low male families are 45% male. These ratios are quite similar to the values I found in 8 female and 13 hermaphrodite-derived sibships in which all individuals were sexed and mortality was kept to a minimum (Petraitis,

1985). Broods from hermaphrodite X male matings are 98% male and from female X male matings are 47% male. The six "females" out of the 297 offspring from hermaphroditic mothers all secondarily developed male copulatory setae. The simplest explanation for these sex ratios is that sex determination in *Capitella* sp. I is a two-factor system in which the females are the heterogametic sex (Petraitis, 1985).

Since the development of hermaphroditism is clearly inhibited by females, it is possible hermaphroditism in *Capitella* is an adaptation to mate competition. *Capitella* quickly colonizes newly disturbed habitats (*e.g.*, Sanders *et al.*, 1980), and it is likely small local populations may be highly synchronized. *Capitella* sp. I reduces sexual maturity in 4 to 6 weeks at 15°C and females require 10 to 14 days to brood eggs to larvae. Thus in synchronized patches the ratio of receptive females to males may decline quite rapidly and remain low for several weeks. Males that can develop eggs and function as females would be at an advantage. In larger populations the probability of synchrony would be lower and thus the occurrence of hermaphrodites much rarer.

In fact, hermaphrodites function more as females than males. Holbrook (1982) examined mating success of hermaphrodites under different total densities and proportions of hermaphrodites. She claims that a hermaphrodite's ability to mate as a male and a female depends on total density and frequency of hermaphrodites (Holbrook, 1982; her Fig. 21). Unfortunately she did not take into account the number of matings that would be expected if all worms within a replicate bowl mated at random. When corrected for this expectation there is no effect of density or frequency, however hermaphrodites avoid mating as males (*i.e.*, there are fewer matings by hermaphrodites as males than would be expected under random mating).

Because the original colonists of a patch may be related and since a patch can persist for several generations, local mate competition may play a role. It is very likely that some patches are founded by siblings since when a mother deserts a brood tube with newly emerging larvae, some larvae remain trapped in the tube. If the tube is carried intact to a new location, then the founders would be siblings.

In this situation, the sex ratio will be biased in favor of the sex which has the least amount of competition among kin (Bulmer and Taylor, 1980a, b; Taylor, 1981). Even when the original colonists are unrelated, the bias depends on the number of original colonists and the number of generations the subpopulation is isolated (Bulmer and Taylor, 1980b).

Two observations suggest that male *Capitella* within a patch may be related. First, patches may be founded by a few individuals and persist for long periods (Sanders *et al.*, 1980). Second, founders may be related if intact brood tubes provide a major method of dispersal.

Yet, *Capitella* does not have a female biased sex ratio as predicted by local mate competition (Petraitis, 1985). The lack of bias is based, however, on the assumption *Capitella* is a species with only females and males. In fact data on sex determination in *Capitella* suggest there are only two sexual genotypes *i.e.*, female (ZW) and male or hermaphrodite (ZZ). Under this sexual system the sex ratio should be strongly biased in favor of hermaphrodites (Lewis, 1941; Charnov, 1982). Since *Capitella* shows nearly a 50:50 sex ratio, this must be interpreted as a female biased sex ratio as predicted by local mate competition.

The two mechanisms, synchronized mating with a decline in receptive females and local mate competition among kin, are not mutually exclusive. Both could be important and both predict that hermaphrodites should function primarily as females. Since female *Capitella* do not develop into hermaphrodites when population

levels are low and since hermaphrodites do not self-fertilize (Grassle, 1980), it seems unlikely that hermaphroditism in *Capitella* is an adaptation for periodic declines in population or for ease of colonization of new habitat. Rather, hermaphroditism in *Capitella* appears to be an adaptation to small local populations which are highly age-structured and in which individuals are related.

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